(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 January 2003 (09.01.2003)

PCT

(10) International Publication Number
WO 03/001981 A2

(51) International Patent Classification[7]: A61B

(21) International Application Number: PCT/US02/20550

(22) International Filing Date: 28 June 2002 (28.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/302,258 29 June 2001 (29.06.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DE, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/001981 A2

(54) Title: METHOD OF PROMOTING ENGRAFTMENT OF A DONOR TRANSPLANT IN A RECIPIENT HOST

(57) Abstract: A method of promoting engraftment of a donor transplant in a recipient host, comprising adoptive transfer to the recipient host donor natural killer (NK) cells, which have been treated *ex vivo* to interfere with the ability of inhibitory receptors on the donor NK cells to interact with major histocompatibility complex molecules in the recipient host, simultaneously with, or sequentially to, in either order, the donor transplant, whereupon the engraftment of the donor transplant in the recipient host is promoted.

METHOD OF PROMOTING ENGRAFTMENT OF A DONOR TRANSPLANT IN A RECIPIENT HOST

FIELD OF THE INVENTION

This invention pertains to a method of using donor natural killer (NK) cells to promote engraftment of a donor transplant in a recipient host, wherein the donor NK cells have been treated *ex vivo*, such as with an antibody (or antigenically reactive fragment thereof), a major histocompatibility molecule (MHC) (or fragment thereof), a small molecule, a blocker of cell-signaling or an enzyme, such that the ability of the donor NK cells to interact with MHC molecules in the recipient host is compromised.

BACKGROUND OF THE INVENTION

Transplants, such as bone marrow transplants, from a donor to a recipient host can be used to treat various non-neoplastic and neoplastic diseases. For example, bone marrow transplants are currently used to treat hematologic malignancies, and solid tumors that remain sensitive to high-dose myeloablative agents, but not conventional doses of chemotherapy. However, there are four obstacles which limit the efficacy of transplant techniques: occurrence of graft-versus-host disease, failure of the transplant to engraft, susceptibility of patients to opportunistic infections after the transplant, and recurrence of neoplastic disease when the transplantation technique is used to treat cancer. Asai *et al.*, "Suppression of Graft-versus-Host Disease and Amplification of Graft-versus-Tumor Effects by Activated natural Killer Cells after Allogeneic Bone Marrow Transplantation," *J. Clin. Invest.* 101(9): 1835-42 (1998).

Graft-versus-host disease (GVHD) causes many problems for the recipient host. It is caused by an attack of donor T-cells against alloantigens of the recipient. Often, GVHD is accompanied by profound immune suppression, which can be life-threatening. Although the incidence of acute GVHD may be diminished by removing the donor T-cells from the donor transplant, T-cell depletion is also associated with increased incidences of disease relapse and failure of the donor transplant to engraft. See, Asai *et al.*, *supra*.

Donor transplants also give rise to graft-versus-tumor (GVT) effects. This may be particularly useful when a transplant procedure is used to treat a cancer if the GVHD effects can be controlled. However, many of the current means employed to control GVHD (i.e. immunosuppression) also inhibit GVT effects. See, Asai *et al.*, *supra*.

Host NK cells represent an important arm of innate immunity and are thought to play a critical role in the immune surveillance against tumors and virally-infected cells.

Studies in mice with severe combined immune deficiency (SCID) demonstrated that NK cells alone could mediate the specificity of bone marrow cell rejection.

Donor NK cells have demonstrated marked beneficial effects for engraftment of donor transplants in a recipient host. In the context of allogeneic bone marrow transplantation, treatment of a transplant recipient host with activated NK cells increases engraftment and promotes donor-cell derived immune reconstitution. Murphy *et al.*, "Donor type activated natural killer cells promote marrow engraftment and B cell development during allogeneic bone marrow transplantation," *J. Immunol.* 148(9): 2953-2960 (1992).

However, depending upon the time at which the donor NK cells are transferred to the recipient host, the donor NK cells may have a protective or deleterious effect. When transferred shortly after allogeneic transplantation, donor NK cells have been shown to suppress the sensitization stage of GVHD during which donor T-cells are first reacting with alloantigens of the recipient. See, Asai *et al.*, *supra*. It is at this point in time that donor NK cells can inhibit GVHD by inhibiting T-cell responses, which may occur, in part, through the production of TGF-β. Furthermore, it has been shown that donor NK cells, transferred at this time, promote donor engraftment, and GVT effects after allogeneic bone marrow transplantation in mice. However, once T-cells become primed and expand, transfer of NK cells to the recipient host results in the production of inflammatory cytokines that can lead to deleterious effects with respect to the transplant. See, Asai *et al.*, *supra*. Transfer of NK cells at this time gives rise to some GVT effects and predominantly GVHD effects.

Furthermore, donor NK cell cytolytic activity may be inhibited by the recipient host. MHC class I antigen expression levels on tumor cell lines have been shown to correlate inversely with susceptibility to NK cell lysis. Gumperz *et al.*, "Specificity of two anti-class I HLA monoclonal antibodies that block class I recognition by the NKB1 killer cell inhibitory response," *Tissue Antigens* 48: 278-284 (1996). For example, bone marrow from β2 microglobulin knockout mice (deficient in MHC class I expression) was universally rejected as a source for donor transplant. A family of MHC class I-specific inhibitor-binding receptors has been characterized, explaining these results.

NK cells express receptors specific for MHC class I determinants. For example, in mice, the Ly49 family of receptors is composed of type II integral membrane homodimers that are C-type lectins and recognize class Ia molecules H2D and/or H2K. A further example is the KIR or killer cell immunoglobulin-like receptors in humans, which are members of the immunoglobulin superfamily (with 2 or 3 loops in the extracellular domain) and recognize class Ia HLA-C, B or A molecules. The majority of these inhibitory receptors have an immunoreceptor tyrosine-based inhibitory motif

(ITIM), which results in a potent inhibitory signal being sent to the NK cell upon binding of the appropriate MHC determinant. Thus, when MHC class I molecules bind to inhibitory receptors on an NK cell, the NK cell is no longer able to promote engraftment of the donor transplant in the recipient host, and no longer has anti-GVHD or pro-GVT effects.

In view of the above, it is an object of the present invention to provide a method of promoting engraftment of a donor transplant in a recipient host. This and other objects and advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a method of promoting engraftment of a donor transplant in a recipient host. The method comprises adoptively transferring to the recipient host donor NK cells, which have been treated *ex vivo* to interfere with the ability of inhibitory receptors on the donor NK cells to interact with MHC molecules in the recipient host, simultaneously with, or sequentially to, in either order, the donor transplant, whereupon the engraftment of the donor transplant in the recipient host is promoted.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method to promote the engraftment of a donor transplant in a recipient host. The method comprises adoptively transferring to the recipient host donor NK cells, which have been treated *ex vivo* to interfere with the ability of inhibitory receptors on the donor NK cells to interact with MHC molecules in the recipient host, simultaneously with, or sequentially to, in either order, the donor transplant. By "promote" is meant a decrease in the frequency of non-engraftment of the donor transplant in the recipient host or to increase the efficiency of engraftment. By "engraftment" is meant the successful uptake of a donor transplant by a recipient host (as "successful uptake" would be understood to one skilled in the medical arts in the context of transplantation). By "donor" is meant a clinically suitable source of material for use as a donor transplant. By "donor transplant" is meant tissue, an organ, bone marrow, or other matter removed from the donor to be transplanted into a recipient host. By "recipient host" is meant an animal, such as a mammal, in particular a human, into which the donor transplant is to be transplanted.

Blocking inhibitory receptors' ability to interact with inhibitory molecules by treating the NK cells *ex vivo* as herein described is beneficial in several respects over currently available techniques. Since the NK cells are no longer inhibited, less NK cells

are required to promote engraftment of a donor transplant in a recipient host. Since the inhibition of the NK cells is compromised, efficiency of engraftment of the donor transplant into the recipient host is also increased. For example, in a preferred embodiment, less bone marrow is required for transplantation to ensure successful engraftment of the bone marrow in the recipient host.

In mice, NK cells can be isolated from any suitable source. Preferably, the NK cells are isolated from single-cell suspensions of splenocytes and red cell lysis of bone marrow cells. Relatively pure populations of NK cells can be propagated in NK cell media (Roswell Park Memorial Institute (RPMI) 1640 media supplemented with 10% fetal bovine serum [FBS], 100 U/mL penicillin/streptomycin, 2 mM L-glutamine, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1 mM nonessential amino acids, 1 mM sodium pyruvate, 2.5×10^{-5} M 2-mercaptoethanol and 1 μg/mL indomethacin) containing 5,000 IU/mL recombinant human interleukin-2 (rhIL-2) at 0.5×10^6 to 1×10^6 cells/ml for up to 14 days.

NK cells also can be isolated from any suitable source in humans. The preferred source of NK cells in humans is peripheral blood. Alternatively, NK cells can be obtained from other sources, such as bone marrow or cord blood. Preferably, the NK cells are isolated from the source by use of flow cytometry. Alternatively, contaminating cells can be removed using positive and negative selection. For example, cell markers such as NK1.1 and DX5 are present on NK cells, as well as on certain T-cell subsets and other cell types. The contaminating cell types can be identified by such markers as CD3 and CD4, which are not present on NK cells. Relatively pure populations of NK cells can be propagated in NK cell media. Resting cells can be frozen and clones can be grown almost indefinitely. The cells can be activated by growing in NK cell media containing 5,000 IU/ml rhIL-2 at 0.5×10^6 to 1×10^6 cells/ml for up to 14 days.

Treatment of donor NK cells *ex vivo* can be done so in accordance with methods known in the art. Desirably, the donor NK cells are treated *ex vivo* so as to interfere with the ability of inhibitory receptors on donor NK cells to interact with MHC molecules in the recipient host. Preferably, the donor NK cells are treated by contact with an antibody (or an antigenically reactive fragment thereof) to an inhibitory receptor on the donor NK cells, an MHC molecule (or a fragment thereof) that binds to an inhibitory receptor on the donor NK cells, a small molecule that binds to an inhibitory receptor on the donor NK cells, a blocker of cell-signaling to or from an inhibitory receptor on the donor NK cells, or an enzyme that modifies an inhibitory receptor on the donor NK cells.

Methods of producing polyclonal antibodies (pAbs) and monoclonal antibodies (mAbs) for specific proteins are well-known in the art. See, e.g., George *et al.*, *J. Immunol.* 163: 1859-1867 (1999); U.S. Patent Nos. 5,786,160, 5,965,401 and 6,194,549 B1; and Example 1 herein. Antibodies also can be purchased from a wide variety of commercial sources. mAbs that bind to an inhibitory receptor on NK cells and block binding to MHC class I molecules are known. See, Gumperz *et al.*, *Tissue Antigens* 48: 278-284 (1996). A recipient host and donor must be typed (as the term "typed" is understood in the medical arts pertaining to hematological screening) to determine which type of MHC class I inhibitory receptors are present, so that an appropriate antibody can be selected to interfere with the ability of inhibitory receptors on donor NK cells to interact with MHC molecules in the recipient host.

An antigenically reactive fragment of an antibody, such as an $F(ab')_2$ fragment of an mAb, also can be used in the context of the present inventive method. $F(ab')_2$ fragments of an mAb can be produced by treating the appropriate mAb with a peptide-digesting enzyme, such as pepsin. The fragments then can be separated and characterized, as desired, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), for example.

The donor NK cells can be contacted with the antibody (or antigenically reactive fragment thereof) by co-incubation at 37 °C for 2 hours, for example, and then washed and suspended in a composition for delivery to the recipient host as herein described, *infra*. Effective blocking of MHC class I molecules to inhibitory receptors on the NK cells can be assayed by a variety of immunological assays. For example, *in vitro* cytotoxicity assays can be performed by measuring the rate at which *ex vivo* treated NK cells kill target cells. See, e.g., Gumperz *et al.*, *supra*, and Asai *et al.*, *supra*. If inhibitory binding is compromised, then NK cell cytotoxicity towards the target cells should be high. If inhibitor binding is not compromised, then low levels of cytotoxicity are expected.

MHC molecules for use in the context of the present inventive method include any suitable MHC molecules, such as those that fit into the active site of the inhibitory receptor on an NK cell, particularly those that interact strongly with the active site such that interaction between the MHC molecules and the inhibitory receptor's active site is preferred over the interaction between an MHC class I molecule and the inhibitory receptor. Similarly, fragments of these MHC molecules can be used in the context of the present inventive method to interfere with the inhibitory receptor's ability to bind to an inhibitory MHC class I molecule. Such fragments of an MHC molecule may be obtained by treating the MHC molecule with a peptide-digesting enzyme, such as pepsin. Desirably, these MHC molecules, or fragments thereof, will not activate the

inhibitory receptor. However, any MHC molecule, or fragment thereof, that does not completely inhibit NK cell activity upon binding to the inhibitory receptor's active site can be selected for use in the context of the present inventive method. The ability of an MHC molecule, or a fragment thereof, to compromise the ability of an inhibitory molecule's ability to interact with the inhibitory receptor can be assayed by a variety of immunological assays. For example, *in vitro* cytotoxicity assays can be performed as described, *supra*.

Small molecules for use in the context of the present inventive method include any suitable small molecules, such as those that fit into the active site of the inhibitory receptor on an NK cell, particularly those that interact strongly with the active site such that the interaction between the small molecule and the inhibitory receptor's active site is preferred over the interaction between on MHC class I molecule and the inhibitory receptor. Other suitable small molecules include those small molecules that interact with the receptor to block binding of an inhibitory molecule to the inhibitory receptor on an NK cell. One method of determining the binding of a small molecule to the inhibitory receptor is by use of a conjugation assay. In such an assay, the NK cells are treated *ex vivo* with a small molecule. The NK cells are then incubated with the target cells which have MHC class I expression. If the small molecule compromises the ability of the MHC class I molecule to bind to the inhibitory receptor, then the NK cell will be unable to bind to the target cell. If the small molecule is not suitable for this purpose, then NK cell binding to the target cell will be detectable. Such conjugation assays are well-known in the art.

Blockers of cell-signaling to or from an inhibitory receptor on donor NK cells also can be used to treat the donor NK cells *ex vivo*. Phosphatase inhibitors, such as pervanadate, can be suitable for blocking the signaling cascade that occurs once an inhibitory molecule binds to the inhibitory receptor. By blocking this signal cascade, the NK cell will not be inhibited by an MHC class I molecule binding to the receptor, as the response mechanism will not receive an inhibitory signal. Suitable cell-signaling inhibitors can be determined by assaying the cytotoxic activity of the NK cells *in vitro*.

Enzymes which modify the inhibitory receptor in such a way as to inhibit the binding of an MHC class I molecule to an inhibitory receptor also can be used to treat the donor NK cells *ex vivo*. For example, the binding of an MHC class I molecule to an inhibitory receptor on a donor NK cell can be a zinc-dependent process. Enzymes which chelate zinc would inhibit binding of an MHC class I molecule to an inhibitory receptor on the donor NK cell. Other suitable enzymes can be assayed using a conjugation assay as described, *supra*.

More than one of the above-described agents can be used, in concert, to treat the donor NK cells *ex vivo* to compromise the ability of inhibitory receptors on the donor NK cells to interact with inhibitory molecules. For example, the donor NK cells can be treated *ex vivo* with one or more of an antibody to an inhibitory receptor on the donor NK cells, an antigenically reactive fragment of an antibody to an inhibitory receptor on the donor NK cells, an MHC molecule that binds to an inhibitory receptor on the donor NK cells, a fragment of an MHC molecule that binds to an inhibitory receptor on the donor NK cells, a small molecule that binds to an inhibitory receptor on the donor NK cells, a blocker of cell-signing to or from an inhibitory receptor on the donor NK cells, and an enzyme that modifies an inhibitory receptor on the donor NK cells to compromise the ability of the inhibitory receptor on the donor NK cells to bind to an inhibitory molecule. Preferably, the donor NK cells are treated *ex vivo* by contacting the inhibitory receptor with an antibody or antigenically reactive fragment thereof and a blocker of cell-signaling. In another preferred embodiment, the donor NK cells are treated *ex vivo* by contact with an MHC molecule or a fragment thereof and a blocker of cell-signaling. In yet another preferred embodiment, the donor NK cells are treated *ex vivo* by contact with a small molecule and a blocker of cell-signaling. In still yet another preferred embodiment, the donor NK cells are treated *ex vivo* by contact with an enzyme that modifies an inhibitory receptor on the donor NK cells and a blocker of cell-signaling.

The NK cells can then be delivered to the recipient host together with, or sequentially to, in either order, a donor transplant. Preferably, the donor NK cells that have been treated *ex vivo* are transferred to the recipient host sequentially after the transplant, such as shortly after the transfer of the donor transplant and before the T-cells become primed and expand (which generally occurs around seven to fourteen days). More preferably, the donor NK cells are transferred to a recipient host within about twenty-four hours to about forty-eight hours after the transplant. Such delivery must occur, however, no later than about five days to about seven days after the transplant (i.e., before the T-cells become primed and expanded). Alternatively and also preferably, the NK donor cells that have been treated *ex vivo* are transferred to the recipient host a short time prior to the transplant. More preferably, the donor NK cells are transferred to a recipient host within about 4 hours to about 12 hours before the transplant. Also alternatively and still preferably, the donor NK cells that have been treated *ex vivo* are transferred to the recipient host together with the transplant. The *ex vivo* treated NK cells also can be transferred to the recipient host at more than one time, together with, or sequentially to, in either order, the transplantation of a donor transplant into the recipient host.

The amount of NK cells to be transferred to the recipient host in context of the present inventive method is determined by the size of the donor transplant. Where the present inventive method is applied to the transplant of bone marrow from a donor to a recipient host, methods of determining the amount of bone marrow to be transferred to a recipient host are known in the art and are dependent upon several factors including the age, weight, and sex of the recipient host, among other factors as are known in the art.

Any suitable route of administration as is known in the art can be used. Likewise, any suitable biocompatible carrier can be used as known in the art. In a preferred embodiment, the donor NK cells treated *ex vivo* to compromise the ability of the inhibitory receptors on the donor NK cells to interact with inhibitory molecules are given intravenously. In another preferred embodiment, the donor NK cells treated *ex vivo* to compromise the ability of the inhibitory receptors on the donor NK cells to interact with inhibitory molecules are injected intraperitoneally. Preferably, the NK cells are suspended in a buffered saline solution, such as phosphate-buffered saline (PBS) or Hunt's buffered salt solution (HBSS).

The NK cells delivered to the recipient host may be activated (by, e.g., recombinant human interleukin- (rhIL-) 2, interleukin-2 (IL-2), IL-15, or rhIL-15), or resting. Activated cells may give rise to increased activity promoting engraftment of a donor transplant in a recipient host relative to the use of resting cells. However, resting cells may give rise to prolonged engraftment-promoting effects relative to the use of activated cells. Use of either activated or resting NK cells treated *ex vivo* as described constitute preferred embodiments of the present invention. A mixture of activated and resting NK cells treated *ex vivo* as described, *supra*, can be delivered as a further embodiment of the present invention.

The present inventive method has application in the context of the transplantation of a variety of tissues from the donor to the recipient host. In a preferred embodiment, the donor transplant is bone marrow. In an alternative embodiment, the donor transplant is an organ. Preferably, the donor and the recipient hosts are mammals. More preferably, either of the donor or the recipient host is a human. Even more preferably, the donor and the recipient host are mammals of the same species. Most preferably, the donor and the recipient host are both humans.

In view of the above, the present invention also provides a method of promoting engraftment in a recipient host comprising:

(i) administering to the recipient host an agent that interferes with the ability of inhibitory receptors on the donor NK cells to interact with MHC molecules in the recipient host,

(ii) simultaneously or subsequently administering to the recipient host donor NK cells, and

(iii) subsequently administering to the recipient host the donor transplant, whereupon the engraftment of the donor transplant in the recipient host is promoted. "Promote," "engraftment," "donor" and "recipient host" are as defined above. NK cells can be isolated from any suitable source, also as indicated above.

"An agent that interferes with the ability of inhibitory receptors on the donor NK cells to interact with MHC molecules in the recipient host" can be any suitable agent. Preferably, the agent is an antibody to an inhibitory receptor on the donor NK cells or an antigenically reactive fragment of an antibody to an inhibitory receptor on the donor NK cells, such as an $F(ab')_2$ fragment, an MHC molecule that binds to an inhibitory receptor on the donor NK cells or a fragment thereof, a small molecule that binds to an inhibitory receptor on the donor NK cells, or a combination thereof, all as described above. The agent that interferes with the ability of inhibitory receptors on the donor NK cells to interact with MHC molecules in the recipient host can be administered by any suitable route. A preferred route of administration is intravenous administration. In this regard, it is important to point out that while the agent is preferably administered prior to or simultaneously with the donor NK cells, in some instances it can be desirable to administer the agent again after the donor NK cells have been administered. The subsequently administered agent can be administered once, twice or even more times. As an alternative to intermittent administration, the agent can be administered continuously from step (i) through step (iii), and even subsequently, as needed to promote engraftment.

Administration of donor NK cells and the transplant are as described above. In this regard, the amount of NK cells to be transferred and the route of administration of NK cells to be used are as described above. Similarly, the NK cells can be optionally activated as described above.

Like the *ex vivo* method, the *in vivo* method described immediately above has application in the context of the transplantation of a variety of tissues from the donor to the recipient host. In a preferred embodiment, the donor transplant is bone marrow. In an alternative embodiment, the donor transplant is an organ. Preferably, the donor and the recipient hosts are mammals. More preferably, either of the donor or the recipient host is a human. Even more preferably, the donor and the recipient host are mammals of the same species. Most preferably, the donor and the recipient host are both humans.

EXAMPLE

The following example further illustrates the invention, but, of course, should not be construed in any way as limiting its scope.

Example 1

This example demonstrates that inhibitory receptor blockade can promote donor transplant engraftment *in vivo*.

Antibodies and Generation of F(ab')$_2$ Fragments

F(ab')$_2$ fragments of normal mouse immunoglobulin-G (NMG) were purchased from Jackson ImmunoResearch (West Grove, PA). F(ab')$_2$ fragments of antibodies raised to mouse Ly49C and I (5E6) and antibodies raised to Ly49G2 (4D11, rat immunoglobulin-G2a) were prepared as previously described by George *et al.*, *supra*. The F(ab')$_2$ fragments were chosen as these fragments constitute the antigen-binding domains of the antibody. The antibodies were purified from ascites fluid by affinity column purification, concentrated to 5 mg/mL, digested with pepsin (protease to create fragments of monoclonal antibodies), and neutralized with 2 M Tris base solution. The neutralized digestion mixture was dialyzed against phosphate-buffered saline (PBS) overnight. The efficiency of digestion and purity of the resulting F(ab')$_2$ fragments were checked by 4%- to 20%-gradient SDS-PAGE.

Donor Chimerism Assay

BALB/c mice were lethally irradiated and injected with B6 severe combined immunodeficient (SCID) donor-type ($H2D^b$ haplotype) NK cells treated *ex vivo* with monoclonal antibodies. One set of NK cells was treated *ex vivo* with the F(ab')$_2$ fragment of a monoclonal antibody to normal rat immunoglobulin-G (NRG), and another set of NK cells was treated *ex vivo* with the F(ab')$_2$ fragment of the 4D11 monoclonal antibody (i.e., an antibody which recognizes the inhibitory receptors). Control mice were injected with PBS or IL-2. Then, the mice were injected with B6 bone marrow cells. Twenty-eight days after bone marrow transplantation, thymocytes (from the thymus) were analyzed for the level of donor-derived cells by assessing the level of donor MHC class I ($H2D^b$) expression by flow cytometry. At twenty-eight days after bone marrow transplantation, splenocytes (from the spleen) were also analyzed for the level of donor-derived T-cells by assessing the level of donor MHC class I ($H2D^b$) expression by flow cytometry. Mice receiving donor-type NK cells treated with 4D11 F(ab')$_2$ fragment showed significantly increased donor chimerism (i.e., number of donor-derived T cells) in both thymocytes and splenocytes without increased GVHD as compared to the mice treated with the donor NK cells treated with NRG F(ab')$_2$ fragment, or the control mice.

This example illustrates that treatment of adoptive transfer of NK cells treated *ex vivo* with the $F(ab')_2$ fragment of a monoclonal antibody to the inhibitory receptor on a donor-type NK cell increases the survival rate of mice after allogeneic bone marrow transplantation due to the promotion of engraftment of the donor transplant.

All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g. "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations of those preferred embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

WHAT IS CLAIMED IS:

1. A method of promoting engraftment of a donor transplant in a recipient host, which method comprises adoptively transferring to the recipient host donor natural killer (NK) cells, which have been treated *ex vivo* to interfere with the ability of inhibitory receptors on the donor NK cells to interact with major histocompatibility complex (MHC) molecules in the recipient host, simultaneously with or sequentially to, in either order, the donor transplant, whereupon the engraftment of the donor transplant in the recipient host is promoted.

2. The method of claim 1, wherein the donor NK cells have been treated *ex vivo* by contact with an antibody to an inhibitory receptor on the donor NK cells or an antigenically reactive fragment of an antibody to an inhibitory receptor on the donor NK cells.

3. The method of claim 2, wherein the antigenically active fragment of an antibody is an $F(ab')_2$ antibody fragment.

4. The method of claim 1, wherein the donor NK cells have been treated *ex vivo* by contact with an MHC molecule that binds to an inhibitory receptor on the donor NK cells or a fragment of an MHC molecule that binds to an inhibitory receptor on the donor NK cells.

5. The method of claim 1, wherein the donor NK cells have been treated *ex vivo* by contact with a small molecule that binds to an inhibitory receptor on the donor NK cells.

6. The method of claim 1, wherein the donor NK cells have been treated *ex vivo* by contact with a blocker of cell-signaling to or from an inhibitory receptor on the donor NK cells.

7. The method of claim 1, wherein the donor NK cells have been treated *ex vivo* by contact with an enzyme that modifies an inhibitory receptor on the donor NK cells.

8. The method of claim 1, wherein the donor NK cells have been treated *ex vivo* by contact with one or more of an antibody to an inhibitory receptor on the donor NK cells, an antigenically reactive fragment of an antibody to an inhibitory receptor on the donor NK cells, an MHC molecule that binds to an inhibitory receptor on the donor NK

cells, a fragment of an MHC molecule that binds to an inhibitory receptor on the donor NK cells, a small molecule that binds to an inhibitory receptor on the donor NK cells, a blocker of cell-signaling to or from an inhibitory receptor on the donor NK cells, and an enzyme that modifies an inhibitory receptor on the donor NK cells.

9. The method of claim 8, wherein the donor NK cells have been treated *ex vivo* by contact with (i) an antibody or an antigenically reactive fragment thereof and (ii) a blocker of cell-signaling.

10. The method of claim 8, wherein the donor NK cells have been treated *ex vivo* by contact with (i) an MHC molecule or a fragment thereof and (ii) a blocker of cell-signaling.

11. The method of claim 8, wherein the donor NK cells have been treated *ex vivo* by contact with (i) a small molecule and (ii) a blocker of cell-signaling.

12. The method of claim 8, wherein the donor NK cells have been treated *ex vivo* by contact with (i) an enzyme and (ii) a blocker of cell-signaling.

13. The method of claims 1-12, wherein the donor transplant is tissue.

14. The method of claims 1-12, wherein the donor transplant is bone marrow.

15. The method of claims 1-12, wherein the donor transplant is an organ.

16. The method of claims 1-15, wherein the donor and the recipient host are mammals.

17. The method of claim 16, wherein the donor or the recipient host is a human.

18. The method of any of claims 1-15, wherein the donor and the recipient host are of the same species.

19. The method of any of claims 1-15, wherein the donor and the recipient host are mammals of the same species.

20. The method of claim 19, wherein the species is human.

21. A method of promoting engraftment of a donor transplant in a recipient host, which method comprises:

(i) administering to the recipient host an agent that interferes with the ability of inhibitory receptors on the donor NK cells to interact with MHC molecules in the recipient host,

(ii) simultaneously or subsequently administering to the recipient host donor NK cells, and

(iii) subsequently administering to the recipient host the donor transplant, whereupon the engraftment of the donor transplant in the recipient host is promoted.

22. The method of claim 21, wherein the agent that interferes with the ability of inhibitory receptors on the donor NK cells to interact with MHC molecules in the recipient host is an antibody to an inhibitory receptor on the donor NK cells or an antigenically reactive fragment of an antibody to an inhibitory receptor on the donor NK cells.

23. The method of claim 22, wherein the antigenically reactive fragment of an antibody is an $F(ab')_2$ fragment.

24. The method of claim 21, wherein the agent that interferes with the ability of inhibitory receptors on the donor NK cells to interact with MHC molecules in the recipient host is an MHC molecule that binds to an inhibitory receptor on the donor NK cells or a fragment of an MHC molecule that binds to an inhibitory receptor on the donor NK cells.

25. The method of claim 21, wherein the agent that interferes with the ability of inhibitory receptors on the donor NK cells to interact with MHC molecules in the recipient host is a small molecule that binds to an inhibitory receptor on the donor NK cells.

26. The method of claim 21, wherein the agent that interferes with the ability of inhibitory receptors on the donor NK cells to interact with MHC molecules in the recipient host is one or more of an antibody to an inhibitory receptor on the donor NK cells, an antigenically reactive fragment of an antibody to an inhibitory receptor on the donor NK cells, an MHC molecule that binds to an inhibitory receptor on the donor NK cells, a fragment of an MHC molecule that binds to an inhibitory receptor on the donor

NK cells, and a small molecule that binds to an inhibitory receptor on the donor NK cells.

27. The method of any of claims 21-26, wherein the donor transplant is tissue.

28. The method of any of claims 21-26, wherein the donor transplant is bone marrow.

29. The method of any of claims 21-26, wherein the donor transplant is an organ.

30. The method of any of claims 21-26, wherein the donor and the recipient host are mammals.

31. The method of claim 30, wherein the donor or the recipient host is a human.

32. The method of any of claims 21-26, wherein the donor and the recipient host are of the same species.

33. The method of any of claims 21-26, wherein the donor and the recipient host are mammals of the same species.

34. The method of claim 33, wherein the species is human.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 January 2003 (09.01.2003)

PCT

(10) International Publication Number
WO 03/001981 A3

(51) International Patent Classification⁷: A01N 63/00, A61K 35/28, 39/395

(21) International Application Number: PCT/US02/20550

(22) International Filing Date: 28 June 2002 (28.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/302,258 29 June 2001 (29.06.2001) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
27 November 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/001981 A3

(54) Title: METHOD OF PROMOTING ENGRAFTMENT OF A DONOR TRANSPLANT IN A RECIPIENT HOST

(57) Abstract: A method of promoting engraftment of a donor transplant in a recipient host, comprising adoptive transfer to the recipient host donor natural killer (NK) cells, which have been treated *ex vivo* to interfere with the ability of inhibitory receptors on the donor NK cells to interact with major histocompatibility complex molecules in the recipient host, simultaneously with, or sequentially to, in either order, the donor transplant, whereupon the engraftment of the donor transplant in the recipient host is promoted.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/26850

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A01N 63/00; A61K 35/28; A61K 39/395;
US Cl. : 424/93.1, 93.7, 577, 809
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/93.1, 93.7, 577, 809

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,800,539 A (WALLER et al) 01 September 1998 (01.09.1998) (see entire document)	1-34
Y	WO 96/38543 A1 (DIACRIN,INCORPORATED). 05 December 1996 (05.12.96) (see entire document, abstract, pages 1, 3 and 6 in particular)	1-34
Y, P	MURPHY W., et al. Immunobiology of natural killer cells and bone marrow transplantation:merging of basic and preclinical studies. Immunological Reviews, 2001, Vol.181, pages 279-289 (see entire document).	1-34
Y	KOH, C et al., Adoptive cellular immunotherapy: NK cells and bone marrow transplantation. Histology and Histopathology, 2000, Vol.15, pages 1201-1210 (see entire document)	1-34

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Date of the actual completion of the international search	Date of mailing of the international search report
27 November 2002 (27.11.2002)	18 DEC 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Michail A Belyavskyi Telephone No. 703/308-0096

Form PCT/ISA/210 (second sheet) (July 1998)

Continuation of B. FIELDS SEARCHED Item 3:
Medline, Embase, Caplus, Biosis, WEST, USPATFULL.
search term: Murphy; Kuh, C; bennett, M; Romagne, F; bone marrow transplantation; graft rejection; natural killer, inhibitory receptors, GVHD; Ly 49 receptor, KIR receptor.